

# Stem CO<sub>2</sub> efflux and its contribution to ecosystem CO<sub>2</sub> efflux decrease with drought in a Mediterranean forest stand

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## ABSTRACT

The rate of metabolic processes demanding energy in tree stems changes in relation with prevailing climatic conditions. Tree water availability can affect stem respiration through impacts on growth, phloem transport or maintenance of diverse cellular processes, but little is known on this topic. Here we monitored seasonal changes in stem CO<sub>2</sub> efflux ( $F_s$ ), radial growth, sap flow and non-structural carbohydrates in trees of *Quercus ilex* in a Mediterranean forest stand subjected since 2003 to either partial (33%) throughfall exclusion (E) or unchanged throughfall (C).  $F_s$  increased exponentially during the day by an effect of temperature, although sap flow attenuated the increase in  $F_s$  during the day time. Over the year,  $F_s$  also increased exponentially with increasing temperatures, but  $F_s$  computed at a standard temperature of 15 °C ( $F_s^{15}$ ) varied by almost 4-fold among dates.  $F_s^{15}$  was the highest after periods of stem growth and decreased as tree water availability decreased, similarly in C and E treatments. The decline in  $F_s^{15}$  was not linked to a depletion of soluble sugars, which increased when water stress was higher. The proportion of ecosystem respiration attributed to the stems was highest following stem growth (23.3%) and lowest during the peak of drought (6.5%). High within-year variability in  $F_s^{15}$  makes unadvisable to pool annual data of  $F_s$  vs. temperature to model  $F_s$  at short time scales (hours to months) in Mediterranean-type forest ecosystems. We demonstrate that water availability is an important factor governing stem CO<sub>2</sub> efflux and suggest that trees in Mediterranean environments acclimate to seasonal drought by reducing stem respiration. Stem respiratory rates do not seem to change after a long-term increase in drought intensity, however.

## Keywords:

Water deficit  
Respiration acclimation  
Carbohydrate  
Carbon balance  
Forest decline  
Net ecosystem exchange

## 1. Introduction

Stem respiration ( $R_s$ ) is an important component of tree and ecosystem carbon budgets; above ground woody tissues accounting for 5–40% of total annual respiration among different forest ecosystems (Lavigne et al., 1997; Xu et al., 2001; Damesin et al., 2002; Bolstad et al., 2004; Zha et al., 2004; Gaumont-Guay et al., 2006; Acosta et al., 2008; Maseyk et al., 2008; Tang et al., 2008; Wieser et al., 2009; Wang et al., 2010; Guidolotti et al., 2013).  $R_s$  and its contribution to ecosystem respiration may vary over time in relation with the sensitivity of stem growth, photosynthesis, carbohydrate transport or nitrogen mobilization to climatic factors such

as temperature and rainfall. Given that temperature and drought are projected to increase in many regions of the world (Giorgi, 2006), it is crucial to better assess how they will affect  $R_s$  at different temporal scales. Accounting for the influence of temperature and water availability on  $R_s$  will improve estimates of ecosystem respiration and partitioning among ecosystem components, but so far the influence of both climatic factors is largely overlooked in the parameterization of most vegetation models (Smith and Dukes, 2013).

The rate of  $R_s$  increases as temperatures rise within a day or throughout the year, but it is often ignored that the rate of increase is not necessarily the same between the two time scales when making temporal extrapolations (e.g. Damesin et al., 2002; Tang et al., 2008; Wang et al., 2010; Guidolotti et al., 2013). Most vegetation models make the implicit assumption that maintenance respiration of living cells is a constant fraction of gross photosynthesis or just vary with instantaneous temperature similarly through the

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year (Smith and Dukes, 2013). However, the increase in respiration following a long-term rise in temperature is generally lower than that occurring in a shorter period of time because of the thermal acclimation of respiratory metabolism (Atkin and Tjoelker, 2003; Atkin et al., 2005). The depletion of soluble sugars can cause respiration rates to decline in response to sustained hot temperatures, and on the other hand, accumulation of soluble sugars in cold-exposed plants can cause respiration rates to increase (Atkin and Tjoelker, 2003; Atkin et al., 2005; Kruse et al., 2011). This phenomenon of thermal acclimation has been observed in many tree species for leaves and roots, but it has rarely been examined for stems (but see Paembonan et al., 1991; Gansert et al., 2002).

Some field studies have suggested that  $R_s$  declines with decreasing soil water content (Levy and Jarvis, 1998; Nepstad et al., 2002; Stahl et al., 2011), but not others (Zach et al., 2010; Maier, 2001; Guidolotti et al., 2013). Discrepancies may arise from the distinct sensitivity of species to water deficit and the lack of studies examining the relationship between tree water availability and  $R_s$ . The cessation of growth seems to be the main effect of drought on reducing  $R_s$  (Ryan, 1991; Levy and Jarvis, 1998; Nepstad et al., 2002), but drought could also slow the rate of  $R_s$  by reducing phloem transport (see Sevanto et al., 2014), the turgor pressure of cells (Saveyn et al., 2007a,b), the availability of respiratory substrates (Wertin and Teskey, 2008; Maier et al., 2010; Maunoury-Danger et al., 2010), or the energy demand of cell maintenance processes if drought sets in progressively (Atkin and Macherel, 2009). In the long run, coordinated anatomical and functional changes at the tree level induced by drought can affect the rates of respiration of woody tissues. For example,  $R_s$  can be altered if increased drought affects wood density (Bowman et al., 2005). Besides, the sapwood area to leaf area ratio can increase as trees are exposed to increasingly dry conditions through a decline in supported leaf area (e.g. Martin-StPaul et al., 2013). Such change in biomass partitioning is crucial to reduce the possibility to suffer embolism in the long term, but it also modifies the ratio between photosynthetic and non-photosynthetic tissues, which could eventually affect  $R_s$ . Only Nepstad et al. (2002) have investigated how a sustained change in throughfall may affect stem respiratory rates. The authors found no effect of experimental throughfall reduction on stem  $\text{CO}_2$  efflux in a tropical forest.

Measuring stem  $\text{CO}_2$  efflux ( $F_s$ ) is the method most widely used to estimate  $R_s$ , although there are some difficulties to unequivocally link both variables.  $F_s$  may not fully represent the metabolic activity of stem living tissues, and the diurnal and seasonal variation of different physical and physiological factors may affect  $F_s$ , but not  $R_s$ . Some of the problems that bias estimation of  $R_s$  from  $F_s$  are re-fixation of respiratory  $\text{CO}_2$  by bark photosynthesis (e.g. Cernusak et al., 2006), resistance to radial  $\text{CO}_2$  diffusion (e.g. Steppe et al., 2007) and  $\text{CO}_2$  transport through the xylem stream. Part of the  $\text{CO}_2$  respired locally can dissolve in xylem sap and flow upward, reducing radial efflux as sap flow rises (e.g. Teskey et al., 2008). However, despite the complex regulation of stem  $\text{CO}_2$  fluxes (Trumbore et al., 2013), studies that have measured xylem  $\text{CO}_2$  concentration, and axial, radial and storage  $\text{CO}_2$  fluxes in tree stems have estimated that  $F_s$  accounts for a high proportion of  $R_s$ . McGuire and Teskey (2004) estimated that, over 24 h,  $F_s$  accounted for 77, 45 and 83% of  $R_s$  in *Fagus grandiflora*, *Platanus occidentalis* and *Liquidambar styraciflua*, respectively. Teskey and McGuire (2007) later estimated that  $F_s$  was approximately 65% of  $R_s$  in another experiment with *P. occidentalis*. The highest contribution of  $F_s$  to  $R_s$  (88% on average over 24 h) was suggested by Bowman et al. (2005) and Saveyn et al. (2008) for *Dacrydium cupressinum* and *Populus deltoides*, respectively. Therefore,  $F_s$  could be considered as a reasonable and practical proxy of  $R_s$ .

In this experiment we sought to explore how variations in temperature and plant water availability affected  $F_s$  at different

temporal scales in a mature coppice of *Quercus ilex*. Specifically, we sought to answer the following questions: (1) is the diel pattern of  $F_s$  governed by temperature? (2) How seasonal variations in temperature and soil water availability affect  $F_s$ ? (3) Does  $F_s$  change after a long-term increase in drought intensity? To address these questions we measured diel changes in  $F_s$  during a year in trees of *Q. ilex* equipped to measure sap flux and radial growth continuously. Half of the trees were subjected to partial exclusion of throughfall since 2003, which allowed comparing the effects of drought at different time scales. We measured the concentration of nitrogen and non-structural carbohydrates through time as potential causes of variation of  $F_s$ . Finally, we scaled measurements of  $F_s$  to the stand level, and compared the importance of considering different temperature response curves of  $F_s$  across the year vs a single annual temperature response curve.

## 2. Materials and methods

### 2.1. Study area

This study was conducted in a forest stand in the south of France (3°35' E, 43°44' N; 270 m a.s.l.). The stand is dominated by *Q. ilex*, which accounts for more than 90% of tree canopy cover. As of 2009, stem density and basal area were, respectively, 5100 stems  $\text{ha}^{-1}$  and 27.4  $\text{m}^2 \text{ha}^{-1}$ . The high density is related to the full coppicing made in 1942. Climate is Mediterranean. Over the period from 1984 to 2011, mean annual temperature was 13.2°C and mean annual rainfall was 913 mm; during the summer months from June through September mean temperature was 20°C and rainfall was 205 mm. The soil is extremely stony, with 75% of stones in the first 50 cm of soil and 90% below.

### 2.2. Experimental design and stem $\text{CO}_2$ efflux

Measurements of  $F_s$  were made at 1.5 m height in five trees of *Q. ilex* nine times in 2011, at approximately monthly intervals from March to November. From May on, we included five additional trees that have been subjected to a partial exclusion of throughfall since spring 2003. By doing so we aimed at (1) enlarging the range of tree water status during summer, under the expectation that trees subject to throughfall exclusion (*E* trees) had lower water potential than control (*C*) trees; and (2) evaluating a long-term acclimation to increased drought (i.e. differences between treatments at a given level of current-year water stress). The throughfall exclusion system is detailed elsewhere (Limousin et al., 2009; Rodríguez-Calcerrada et al., 2011). Briefly, throughfall is intercepted by PVC rain gutters conducting water outside three 100  $\text{m}^2$  plots. The ground area covered by the gutters is 33%. This makes for an average reduction in net precipitation to the soil of 27% compared to the *C* plots, which are also equipped with gutters turned upside down to balance the understory microclimate in both treatments. Throughfall exclusion has translated into higher cumulative water stress in *E* than *C* trees over the first 6 years of exclusion. Water stress integral during summer months, a parameter resulting from the summation of daily predawn leaf water potential over this period, was 26% lower in *E* than *C* trees (see Rodríguez-Calcerrada et al., 2011). Sampled trees were in the middle of one of the three plots. Mean stem diameter ( $\pm$ SE) at breast height (DBH) at the beginning of the study was  $12.8 \pm 1.1$  cm for *E* trees and  $11.5 \pm 0.6$  cm for *C* trees.

Stem  $\text{CO}_2$  efflux ( $F_s$ ) was measured in situ using a portable infrared gas analyser (LI-6400, Li-Cor Inc., Lincoln, NE, USA) coupled to a LI-6400-09 soil chamber, following the technique and protocol detailed in Xu et al. (2000). Collars of polyvinyl chloride (PVC) were made to assemble the chamber to the stem; one end of the collar



housing the chamber and the other being shaped as to match the curvature of the stem. Loose bark was removed from the surface of measurement and then collars were attached onto the stems by applying 100% silicone sealant. Measurements were made by drawing the CO<sub>2</sub> concentration inside the chamber down and letting it increase from stem CO<sub>2</sub> efflux up to an upper concentration limit. The lower and upper CO<sub>2</sub> concentration limits were changed through the day so as to bracket the ambient air CO<sub>2</sub> concentration, which was on average 380 ppm during the day and 394 ppm during the night. The rate of change of CO<sub>2</sub> over time was computed at ambient CO<sub>2</sub> concentration in three consecutive measurement cycles. Measurements were made at ambient air temperature and relative humidity. Values of  $F_s$  given by the LI-6400 system were re-calculated to account for the volume of the stem collar. To measure this volume, the open end of the PVC collar was closed and water from a graduated cylinder was added through a hole on the top of the collar. A further correction of  $F_s$  per unit surface area was applied to account for the curvature of the stem by using the equation given in Xu et al. (2000).

Stem CO<sub>2</sub> efflux ( $F_s$ ) was measured at six, occasionally five times on each date, typically three times from dusk to dawn and three from dawn to afternoon. Stem temperature ( $T_s$ ) at 2 cm depth was measured every 5 min and averaged at 30-min intervals for each tree, which allowed us to assess the short-term temperature sensitivity of  $F_s$  ( $Q_{10}$ ) through the relationship of  $F_s$  and  $T_s$ . To test for a possible time-lag between these variables, the natural logarithm of  $F_s$  was regressed against  $T_s$  at the time of measuring and at 30, 60, 90 and 120 min earlier, and the coefficients of determination were compared (Ryan et al., 1995; Acosta et al., 2008). From the best regression model, we calculated the rate of  $F_s$  at the prevailing  $T_s$  of each date (i.e. at mean  $T_s$  of the week preceding measurements;  $F_s^{\text{amb}}$ ) and at 15 °C ( $F_s^{15}$ ), a temperature commonly reported in literature and close to the average annual  $T_s$  (14.2 °C). Finally, we calculated the  $Q_{10}$  by using the equation:

$$Q_{10} = e^{10k},$$

with  $k$  being the slope of the linear regression of the natural logarithm of  $F_s$  against  $T_s$  (Atkin et al., 2005).

### 2.3. Sap flow

Xylem sap velocity ( $V_s$ ) was estimated in the same trees sampled for  $F_s$  using two 2-cm-long thermal dissipation probes inserted radially into the stem and separated 10 cm along its vertical axis.  $V_s$  was calculated from the difference in temperature between the upper probe, which was constantly heated, and the lower probe measuring current wood temperature (Granier, 1985; Granier et al., 1992). Data were taken at 5 min intervals and the 30-min means recorded in CR10X data loggers (Campbell Scientific Ltd.). Both probes were shielded from direct sunlight by aluminium reflectors.

The influence of  $V_s$  on  $F_s$  was evaluated by examining the relationship of  $V_s$  with residuals of  $F_s$ , i.e. the ratio of the  $F_s$  observed and that predicted from the regression against  $T_s$  when  $V_s$  was negligible (i.e.  $V_s < 0.01 \text{ mm s}^{-1}$ ; Maier and Clinton, 2006; Hölttä and Kolari, 2009). Furthermore, we compared  $F_s^{15}$  derived from models including either all data or just data at  $V_s < 0.01 \text{ mm s}^{-1}$ .

### 2.4. Tree water availability

Shoot water potential was used as a surrogate of tree water availability. One shoot from each tree in which  $F_s$  was measured was excised before dawn and immediately measured using a pressure chamber (PMS Instrument Company, Albany, OR, USA).

### 2.5. Stem radial growth

Stem diameter was continuously monitored with dendrometer bands (ELPA-93, University of Oulu, Finland) in the same trees where  $F_s$  and  $V_s$  were measured. We computed the rate of diameter increment at each date as the slope of stem diameter vs. time in the one-month period bracketing the measurement of  $F_s$ . We considered  $F_s$  to potentially reflect both growth and maintenance respiration in a given date when significant diameter increments were registered in that period. The only exception was for the date of October, when low  $\Psi_{\text{pd}}$  ( $\approx -4 \text{ MPa}$ ) at the time of  $F_s$  measurements likely precluded any growth-related activity preceding subsequent rains and stem growth.

### 2.6. Chemical analyses

Stem cores were extracted on May, June, late August and October from trees belonging to C and E treatments, although not those in which  $F_s$  was measured to avoid any damage in these trees. Samples were taken at midday and brought to the laboratory for being oven-dried at 60 °C for three days. Bark was easily separated from sapwood. Then outer bark was removed and the concentration of carbon, nitrogen (N) and total non-structural carbohydrates were separately analyzed in the inner bark and the outer 0.5 cm of sapwood. The concentration of carbon and nitrogen was measured using an elemental analyzer (Flash EA1112 Series, Thermo Finnigan, Italy). The concentration of non-structural carbohydrates was measured in a gas chromatograph coupled to a mass spectrometer (GC-MS; Shimadzu QP2010plus). Soluble sugars (SS) were extracted as described in Göttlicher et al. (2006). The supernatant was dried in vacuo (SpeedVac Plus, Savant Instruments, New-York, USA), and then sugars were derivatized following Fiehn (2006). The extraction of starch was made on the pellet resulting from the extraction of sugars. The pellet was washed with 1 ml of 20, 60 and 96% ethanol solutions, dried in vacuo, and then digested at 60 °C with 3U of Amyloglucosidase from *Rhizopus* sp. in sodium acetate buffer. The quantification in the GC-MS was performed relative to internal and external standards; phenyl- $\beta$ -D-glucopyranoside was the internal standard, and starch, D(+)-glucose, fructose and D(+)-sucrose were the external standards. Sugars were identified by their retention times; the identification was further verified by comparing their mass spectral data with the mass spectral library.

### 2.7. Sapwood density

Sapwood density was measured in 40 trees from each treatment in April 2011, excluding those sampled for  $F_s$ . The dry weight of samples were divided by their volume, which was measured using a density determination kit (Sartorius, YDK01, Germany), based on the principle of Archimedes.

### 2.8. Upscaling stem CO<sub>2</sub> efflux to the stand level

Punctual measurements of  $F_s$  expressed as a function of sapwood volume were upscaled to the stand (three 100 m<sup>2</sup> plots) and the 24 h of the sampling days using the following equation:

$$F_{s\text{-stand}} = \frac{\sum_{i=1}^n \sum_{j=1}^{48} F_{si}^{15} Q_{10}^{(T_{sj}^{\text{amb}} - 15)/10} \cdot SV_i}{\text{Total plot area}},$$

where SV means stem sapwood volume (see below), and subscripts  $i$  and  $j$  refer to stems in the plots ( $i = 1, \dots, n$ ) and half hourly temperatures ( $j = 1, \dots, 48$ ), respectively. To assess the importance

of seasonal shifts in respiratory characteristics ( $F_s^{15}$  and  $Q_{10}$ ) in modelling  $F_{s-stand}$ , these parameters were either taken from the temperature response curves of  $F_s$  of each date or the single response curve fitted to all annual data. Furthermore, to improve the accuracy of the up scaling, we examined the relationship between  $F_s$  and diameter in 25 extra trees subjected to normal rainfall during one night in April and August. Stem temperature at measurement time in these trees was calculated by interpolating the 30-min mean of 16 sensors placed in other stems to 1 min intervals. We used the mean  $Q_{10}$  obtained for the trees measured more frequently in the control plot to standardize  $F_s$  rates of the 25 extra trees to a common reference temperature.

The sapwood volume of each stem in the plot was calculated from allometric relationships derived from trees outside the plots. Sapwood area (SA, cm<sup>2</sup>) was measured in 16 cut trees and expressed as a function of DBH (in cm) as:

$$SA = \beta_1 DBH^{\alpha_1},$$

with  $\beta_1 = 0.69$  (95% confidence interval = 0.29–1.08),  $\alpha_1 = 1.89 \pm 0.10$ ,  $r^2 = 0.97$ ,  $n = 16$ . The allometric relationship between height ( $H$ , cm) and DBH was:

$$H = \beta_2 DBH^{\alpha_2},$$

with  $\beta_2 = 128.7$  (95% confidence interval = 121.6–135.8),  $\alpha_2 = 0.57 \pm 0.01$ ,  $r^2 = 0.69$ ,  $n = 891$ . Thus, assuming constant sapwood depth along the stem, sapwood volume (in cm<sup>3</sup>) was calculated as:

$$SV = SA \cdot H = \beta_1 \beta_2 DBH^{(\alpha_1 + \alpha_2)} = 88.7 DBH^{2.465}.$$

### 2.9. Ecosystem respiration

An eddy-covariance flux tower located approximately 100 m away from the C plot provided data of CO<sub>2</sub> ecosystem exchange. Technical details on data acquisition, filtering and partitioning of net ecosystem exchange into gross primary production and ecosystem respiration ( $R_{eco}$ ) can be found in Allard et al. (2008). Briefly, data processing and gap-filling was made based on the methodology developed by the FLUXNET global network (<http://fluxnet.ornl.gov/>). Daytime  $R_{eco}$  was estimated from nighttime data using an algorithm including seasonality in temperature sensitivity of respiration (Reichstein et al., 2005). The estimated value of stand-scale stem CO<sub>2</sub> efflux ( $F_{s-stand}$ ) was compared with  $R_{eco}$  on the nine days of sampling.

### 2.10. Statistical analysis

The main effects of date and treatment and the interaction effect between both factors were tested with General Linear Models (GLM). The date was included in models as a within-subjects factor for analysis of variables that were repeatedly measured on the same stem. Means of treatments were further compared by dates when the interaction between date and treatment factors was significant ( $P < 0.05$ ). Differences between treatments in the relationship between independent (temperature,  $\Psi_{pd}$  and stem growth rate) and dependent ( $F_s^{amb}$ ,  $F_s^{15}$  and  $Q_{10}$ ) variables were tested through the interaction effect between the independent variable and the factor treatment. Some variables were logarithmically transformed to make relationships linear. Analyses were made with Statistica 7.1 (StatSoft, Tulsa, OK, USA).

## 3. Results

### 3.1. Environmental conditions

Air and stem temperatures increased from winter to summer and reached the maximum annual values of 2011 in August (Fig. 1a). The dry period started late because of a rainy month of July and lasted abnormally long due to the absence of significant rainfall until late October, when it rained 578 mm (50% of the annual total) in one month. This caused a clear seasonality in the soil water available to trees ( $\Psi_{pd}$ ; Fig. 1b), and resulted in a lack of correlation between temperature and  $\Psi_{pd}$  across measurement dates which was useful in untangling temperature and  $\Psi_{pd}$  effects on seasonal shifts of stem CO<sub>2</sub> efflux ( $F_s$ ).  $\Psi_{pd}$  was significantly lower for trees subjected to throughfall exclusion, but only from August through October ( $P$ -value of date by treatment interaction effect  $< 0.001$ ).

### 3.2. Diurnal changes in stem CO<sub>2</sub> efflux

On each date of measurement, the proportion of variance in  $F_s$  explained by stem temperature ( $T_s$ ) was low when all trees were pooled together because of between-tree variability in  $F_s$ , but it was high when trees were considered individually. Of the 80 individual curves of  $F_s$  and concurrent  $T_s$  fitted across dates and treatments, 73% showed coefficients of determination ( $r^2$ ) higher than 0.90 and only 6% had  $r^2 < 0.50$ . The goodness of fit increased for 38 curves when  $F_s$  was plotted against the  $T_s$  registered 30–120 min earlier, with 80% of curves having  $r^2$  values higher than 0.90 and only 3% (two curves) having  $r^2 < 0.50$ . As  $\Psi_{pd}$  decreased over time, there was a trend for more trees to have the best  $r^2$  with any of the stem temperatures previous to rather than concurring with  $F_s$  (Fig. 2a). Similarly, the average time lag between  $F_s$  and stem temperature

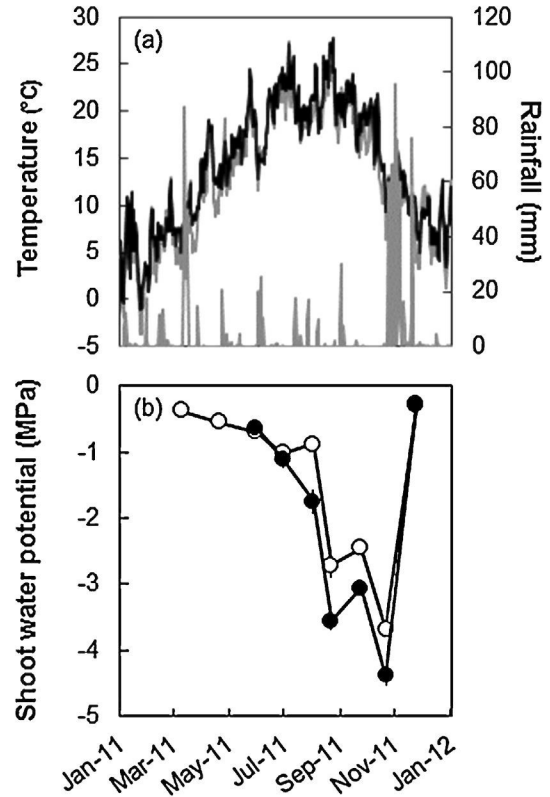
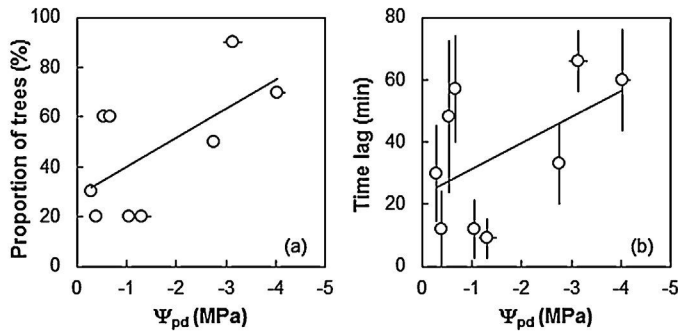
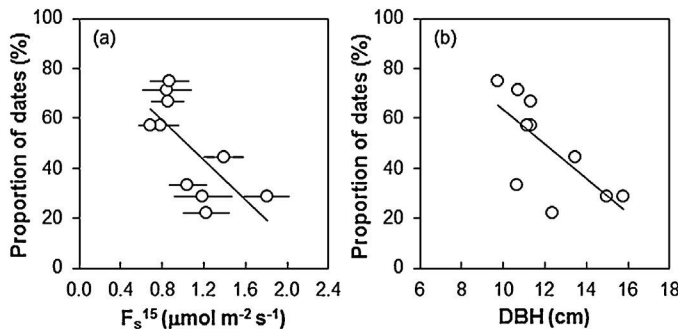


Fig. 1. Seasonal variation of (a) air temperature (grey line), stem temperature (black line) and rainfall (grey line); and (b) predawn shoot water potential in trees subjected to throughfall exclusion and normal throughfall (black and white symbols, respectively).





**Fig. 2.** Relationship between mean predawn shoot water potential ( $\Psi_{pd}$ ) at each date and (a) percentage of sampled trees (5 in the first two dates and 10 in the rest) showing a delayed response of stem  $\text{CO}_2$  efflux ( $F_s$ ) to temperature and (b) mean time lag of  $F_s$  in relation to temperature. Regression models were depicted in (a)  $r^2$ : 0.40,  $n=9$ ,  $P=0.068$ ; and (b)  $r^2$ : 0.27,  $n=9$ ,  $P=0.156$ .



**Fig. 3.** Relationship between (a) mean stem  $\text{CO}_2$  efflux ( $F_s$ ) at  $15^\circ\text{C}$  ( $F_s^{15}$ ) and (b) stem diameter at breast height (DBH) of each tree with the percentage of dates (7–9) in which the tree showed a delayed response of  $F_s$  to temperature. Regression models were depicted in (a)  $r^2$ : 0.49,  $n=10$ ,  $P=0.024$ ; and (b)  $r^2$ : 0.49,  $n=10$ ,  $P=0.023$ .

showed a weak trend to increase with decreasing  $\Psi_{pd}$  ( $r^2$ : 0.27,  $n=9$ ,  $P=0.156$ ; Fig. 2b). Concerning variability among individuals, there was a negative correlation between the annual average rate of  $F_s$  of a tree and the number of dates in which it exhibited a lagged response of  $F_s$  to temperature (Fig. 3a). Further, bigger trees, with higher area-based  $F_s$  (see below), had less frequently a lagged response of  $F_s$  to temperature (Fig. 3b).

In some dates, more noticeably in April, a hysteretic relationship was observed between  $F_s$  and the concurrent stem temperature, so that at a common temperature of  $15^\circ\text{C}$ ,  $F_s$  was higher at times of negligible sap velocity ( $V_s < 0.01 \text{ mm s}^{-1}$ ) than at times with appreciable sap flow (supplementary Fig. S1). To explore whether  $V_s$  modified the relationship between  $T_s$  and  $F_s$ , we calculated the residuals between the values of  $F_s$  observed and those predicted from  $T_s$  when  $V_s < 0.01 \text{ mm s}^{-1}$ ; then we plotted the residuals against  $V_s$ . We found that the residuals varied with  $V_s$ , but in a different way when concomitant stem temperatures were used in the regression models than when the time lag in the response of  $F_s$  to temperature was taken into account (Table 1): the ratio between the observed and predicted values tended to decrease with increasing  $V_s$  within most and across all dates only when concomitant stem temperatures were used in the regression models.

Supplementary Fig. I related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.agrformet.2014.04.012>.

### 3.3. Seasonal changes in stem $\text{CO}_2$ efflux

Stem  $\text{CO}_2$  efflux ( $F_s$ ) increased with increasing  $T_s$  throughout the year (Fig. 4). A close look to the annual response curve evidenced differences in the base rate and temperature sensitivity

**Table 1**

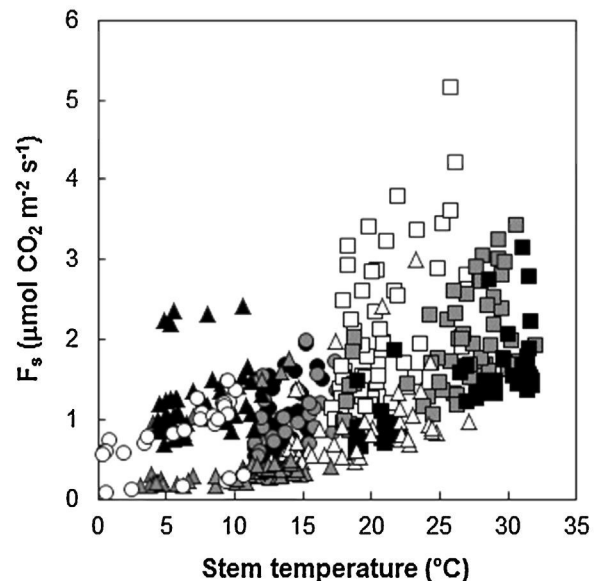
Summary of simple linear regressions between sap velocity ( $V_s$ ) and the ratio between the observed value of stem  $\text{CO}_2$  efflux ( $F_s$ ) and the value predicted from the regression of  $F_s$  against stem temperature at  $V_s < 0.01 \text{ mm s}^{-1}$ . Regressions are derived either from the concomitant stem temperature (Method 1) or the temperature over the preceding 2 h that gave the best fit (Method 2). Analyses are specified by dates for both treatments (control and throughfall exclusion) pooled. Abbreviations:  $r$  (coefficient of correlation) and  $P$  (statistical significance of regression model).

Date	Method 1		Method 2	
	$r$	$P$	$r$	$P$
9 March	−0.29	0.173	−0.14	0.524
20 April	<b>−0.59</b>	<b>0.003</b>	−0.24	0.264
31 May	<b>−0.45</b>	<b>0.002</b>	0.12	0.455
29 June	−0.07	0.6	<b>0.38</b>	<b>0.003</b>
2 August	<b>0.28</b>	<b>0.032</b>	<b>0.51</b>	<b>&lt;0.001</b>
22 August	<b>−0.53</b>	<b>&lt;0.001</b>	−0.03	0.828
23 September	0.21	0.147	−0.04	0.775
21 October	<b>−0.35</b>	<b>0.006</b>	−0.01	0.914
24 November	−0.17	0.267	−0.1	0.48
All data	<b>−0.24</b>	<b>&lt;0.001</b>	0.06	0.207

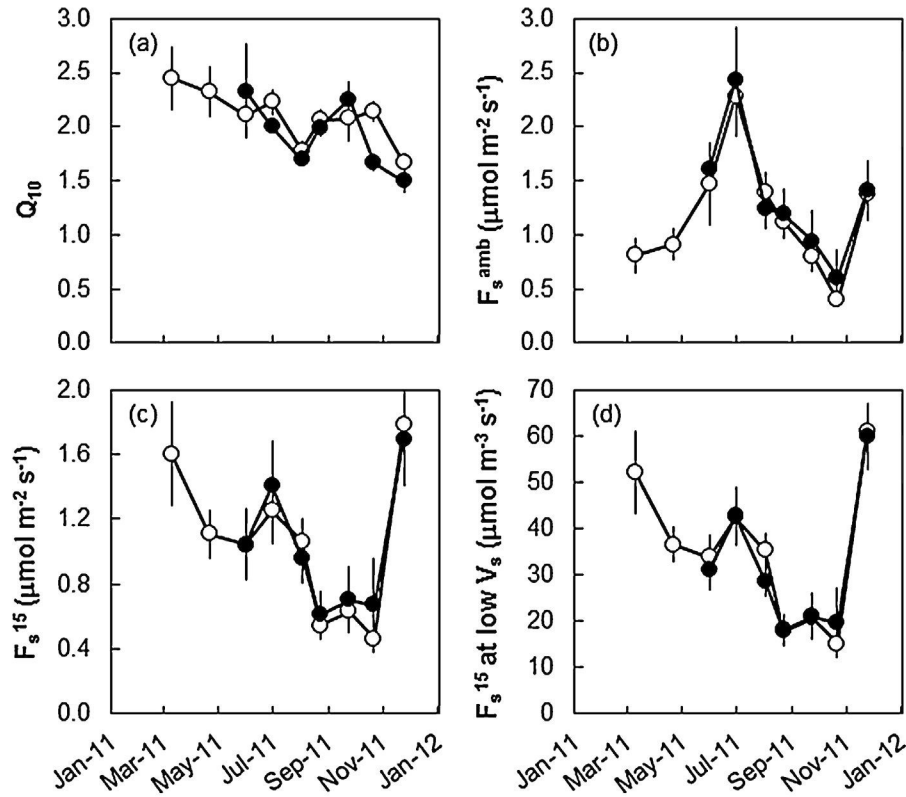
Significant relationships at  $P < 0.05$  are marked in bold characters.

of  $F_s$  between dates. To examine seasonal shifts in respiratory features, individual curves for each stem were used to calculate  $Q_{10}$ ,  $F_s^{\text{amb}}$  and  $F_s^{15}$  at each date, with the time lag between  $F_s$  and  $T_s$  being considered for calculations. The results showed that the values of  $Q_{10}$  varied over the year without a clear pattern (Fig. 5a). Stem  $\text{CO}_2$  efflux at the prevailing stem temperature of each date ( $F_s^{\text{amb}}$ ) peaked by the end of June and again, less markedly, by the end of November (Fig. 5b). At a common stem temperature of  $15^\circ\text{C}$ ,  $F_s^{15}$  showed an additional peak in March (Fig. 5c). There were no differences in  $F_s^{15}$  between throughfall exclusion treatments, whether it was expressed per unit of stem surface area or sapwood volume (see Fig. 5c and d). Similarly,  $F_s^{15}$  obtained from temperature regressions at  $V_s < 0.01 \text{ mm s}^{-1}$  (Fig. 5d) was similar in C and E trees, which suggest differences in  $V_s$  between treatments ( $P < 0.001$ ; Fig. 6a) did not affect the comparison of  $F_s$ . Similar results were found for  $Q_{10}$  and  $F_s^{\text{amb}}$  (not shown).

We examined to what extent seasonal variations in  $F_s^{\text{amb}}$ ,  $F_s^{15}$  and  $Q_{10}$  depended on prevailing temperatures, tree water



**Fig. 4.** Scatter plot of stem  $\text{CO}_2$  efflux ( $F_s$ ) vs. concurrent stem temperature at 2 cm depth separated by sampling dates: 9 March (white circles), 20 April (grey circles), 31 May (black circles), 29 June (white squares), 2 August (grey squares), 22 August (black squares), 23 September (white triangles), 21 October (grey triangles), and 24 November (black triangles).

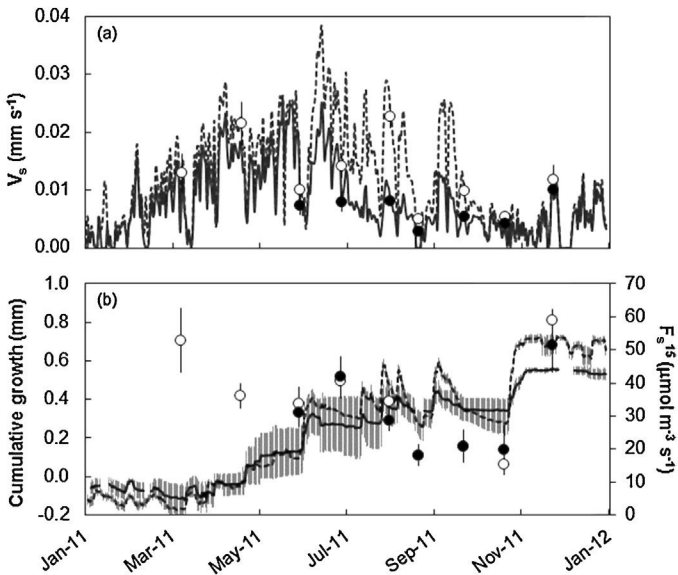


**Fig. 5.** Seasonal variation of (a) sensitivity of stem CO<sub>2</sub> efflux ( $F_s$ ) to diel variations in temperature ( $Q_{10}$ ); (b)  $F_s$  at the prevailing stem temperature of each date ( $F_s^{\text{amb}}$ ); (c)  $F_s$  at 15°C ( $F_s^{15}$ ); and (d)  $F_s^{15}$  derived from temperature response curves of  $F_s$  with only data points of negligible sap velocity ( $V_s < 0.01 \text{ mm s}^{-1}$ ). Values are means ( $\pm$ SE) from five trees subjected to either normal rainfall (white symbols) or partial throughfall exclusion (black symbols). Values of  $F_s$  are expressed as a function of stem surface area (b, c) or sapwood volume (d).

availability, and radial growth rates. There were trends for  $F_s^{\text{amb}}$  to increase ( $r^2: 0.22$ ,  $n = 16$ ,  $P = 0.063$ ; not shown) and  $F_s^{15}$  to decrease (Fig. 7a) as temperatures increased through the year. Tree water availability showed a strong positive relationship with  $F_s^{15}$  (Fig. 7b)

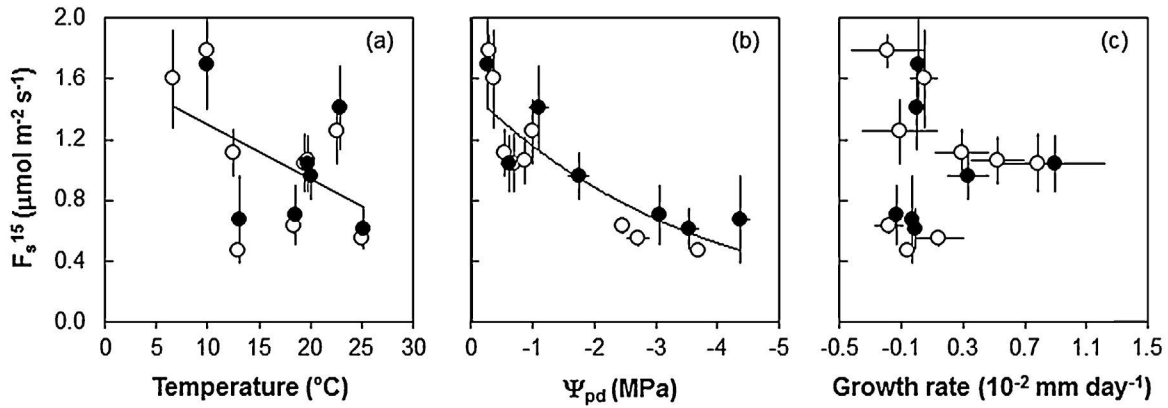
and was also positively correlated with  $F_s^{\text{amb}}$  ( $r^2: 0.28$ ,  $n = 16$ ,  $P = 0.036$ ; not shown), but not with  $Q_{10}$  (not shown). Slopes were not significantly different between treatments at  $P < 0.05$  on any of those cases.

Across dates, stem growth rate was not correlated with  $F_s^{15}$  (Fig. 7c), nor with  $Q_{10}$  or  $F_s^{\text{amb}}$  (not shown). Stem diameter growth started in the first week of May, by day of year (DOY)  $127 \pm 5$  in C and day  $126 \pm 8$  in E; it was negligible in summer and finished in the first week of November, by DOY  $308 \pm 2$  in C and  $311 \pm 2$  in E (Fig. 6b). Stem diameter growth reached  $0.75 \pm 0.10 \text{ mm}$  in C and  $0.57 \pm 0.18 \text{ mm}$  in E over the year of the experiment. The higher rates of growth took place in May and then again in late October, but were uncoupled from  $F_s$  (Fig. 6b). For example, high  $F_s^{15}$  in March occurred 60 days before stem increment took place, and the peak of  $F_s^{15}$  in late November occurred when stem diameter increment was nil. Across trees, however, there were positive correlations between stem growth rate and  $F_s^{15}$  for the dates of May and early August (Fig. 8). The relationship between relative growth rate and  $F_s^{15}$  was not significantly different between dates or treatments ( $P$ -value of interaction effects  $> 0.10$ ), and the linear regression model for all data pooled indicated  $F_s^{15}$  at zero growth was  $0.70 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ . Assuming  $F_s \approx R_s$ , it was estimated that 30% of respiratory costs in May and early August (i.e.  $0.35 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) were attributed to growth, after subtracting the value of  $0.70 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  to the mean  $F_s^{15}$  observed in these dates. From this relationship, we calculated the growth respiration coefficient to be  $0.19 \text{ g C respired g}^{-1} \text{ C wood formed}$ . Density of sapwood was  $0.87 \text{ g cm}^{-3}$  and average carbon concentration 46.6% in the sapwood and 42.8% in the inner bark, with no differences between treatments. These values were used to convert rates of stem diameter increment into carbon accumulation rates and calculate the growth respiration coefficient.



**Fig. 6.** Seasonal variation of (a) average sap velocity ( $V_s$ ) during daytime hours (solar radiation  $> 4 \text{ W m}^{-2}$ ) and (b) cumulative stem diameter increment, in trees subjected to either normal rainfall (white symbols and dashed line) or partial throughfall exclusion (black symbols and continuous lines). Symbols in (a) refer to  $V_s$  in days on which stem CO<sub>2</sub> efflux was measured; in (b) they refer to stem CO<sub>2</sub> efflux at 15°C ( $F_s^{15}$ ). Values are means ( $\pm$ SE) of  $n = 5$  stems.





**Fig. 7.** Relationship between stem CO<sub>2</sub> efflux at 15°C ( $F_s^{15}$ ) and (a) prevailing stem temperature at each measurement date, (b) shoot predawn water potential ( $\Psi_{pd}$ ), and (c) stem diameter increment rate. Values are means ( $\pm 2SE$ ) from nine dates and five trees subjected to either normal rainfall (white symbols) or partial throughfall exclusion (black symbols). Values of  $F_s^{15}$  are expressed as a function of stem surface area. Regression models significant at  $P < 0.10$  were depicted for all data pooled: (a)  $y = 1.66 - 0.04x$ ;  $r^2 = 0.24$ ,  $n = 16$ ,  $P = 0.056$ ; and (b)  $y = 1.52e^{0.27x}$ ;  $r^2 = 0.78$ ,  $n = 16$ ,  $P < 0.001$ .

From the relationship between relative growth rate and  $F_s^{15}$  in early May and August we could not obtain positive estimations of growth  $R_s$  for the rest of dates (assuming  $F_s \approx R_s$  and total  $R_s = \text{growth } R_s + \text{maintenance } R_s$ ). Negative estimations of growth  $R_s$  were also obtained when considering the average  $F_s^{15}$  in dates with no growth as indicative of maintenance  $R_s$ , or after modelling maintenance  $R_s$  as a function of  $\Psi_{pd}$  over the same period. Positive estimations of growth  $R_s$  were only obtained when the lowest annual value of  $F_s^{15}$  (registered at peak drought) was subtracted to total efflux in the rest of dates. This way, the proportion of  $R_s$  attributed to growth processes showed a clear seasonal pattern, being high at the beginning and end of the growing season, and decreasing as drought increased (Supplementary Fig. S2).

Supplementary Fig. II related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.agrformet.2014.04.012>.

### 3.4. Stem chemical composition

The decline in  $F_s^{15}$  through summer and early autumn was not related to low concentration of non-structural carbohydrates (TNC) (Fig. 9). Actually, the concentration of TNC in the sapwood was

significantly higher in October than in May in both treatments, being intermediate in the other dates. The concentration of TNC in the inner bark was the highest in October, but only in the case of C trees ( $P$ -value of date by treatment interaction effect = 0.029). The seasonal increase in the concentration of TNC was due to that in the concentration of soluble sugars because starch tended to decline over time, statistically significantly in the inner bark. To some extent, variations in starch and soluble sugars across dates were correlated with the water status of trees (Fig. 10a and b). The concentration of starch tended to decline and that of soluble sugars to increase as water stress increased. The concentration of nitrogen (N) in the sapwood, which was half that in the inner bark, was significantly lower in October than in August ( $P$ -value of date main effect = 0.037). However, as it occurred for TNC, seasonal changes in  $F_s^{15}$  were not related to variations in N concentration (Fig. 9).

### 3.5. Upscaling stem CO<sub>2</sub> efflux to the stand level

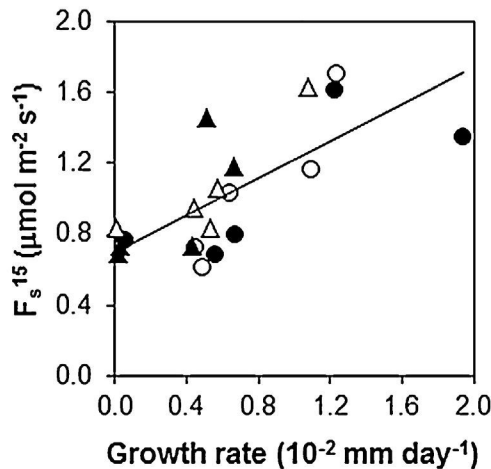
We found no correlation between stem diameter and surface-area-based  $F_s^{15}$  in April but a positive one in August (Fig. 11). When  $F_s$  was expressed per unit of sapwood volume, there was a negative correlation between stem diameter and  $F_s^{15}$  in April, but no correlation in August. Based on these results, we considered the negative relationship between stem diameter and  $F_s^{15}$  in April for modelling stand-scale stem CO<sub>2</sub> efflux ( $F_{s\text{-stand}}$ ) in spring.

Two models of up-scaling were compared: one in which the values of  $Q_{10}$  and  $F_s^{15}$  of each date were applied to calculate  $F_{s\text{-stand}}$  in the respective dates, and another one in which the values of  $Q_{10}$  and  $F_s^{15}$  resulting from the single annual temperature response curve were applied to all dates (Table 2). In the first case,  $F_{s\text{-stand}}$  was the highest in late June and lower in winter and summer, in a seasonal pattern similar to that of  $F_s^{\text{amb}}$ . The proportion of ecosystem respiration ( $R_{\text{eco}}$ ) that was ascribed to  $F_{s\text{-stand}}$  peaked also in late June and declined with increasing water stress (Fig. 10c). The results changed when  $F_{s\text{-stand}}$  in each date was modelled from one single value of  $Q_{10}$  and  $F_s^{15}$ : compared to estimations from the previous model,  $F_{s\text{-stand}}$  was 0.09–0.13 g C m<sup>-2</sup> soil day<sup>-1</sup> higher during the drought period ( $\Psi_{pd} < -1$  MPa) and 0.03–0.27 g C m<sup>-2</sup> soil day<sup>-1</sup> lower in the rest of dates.

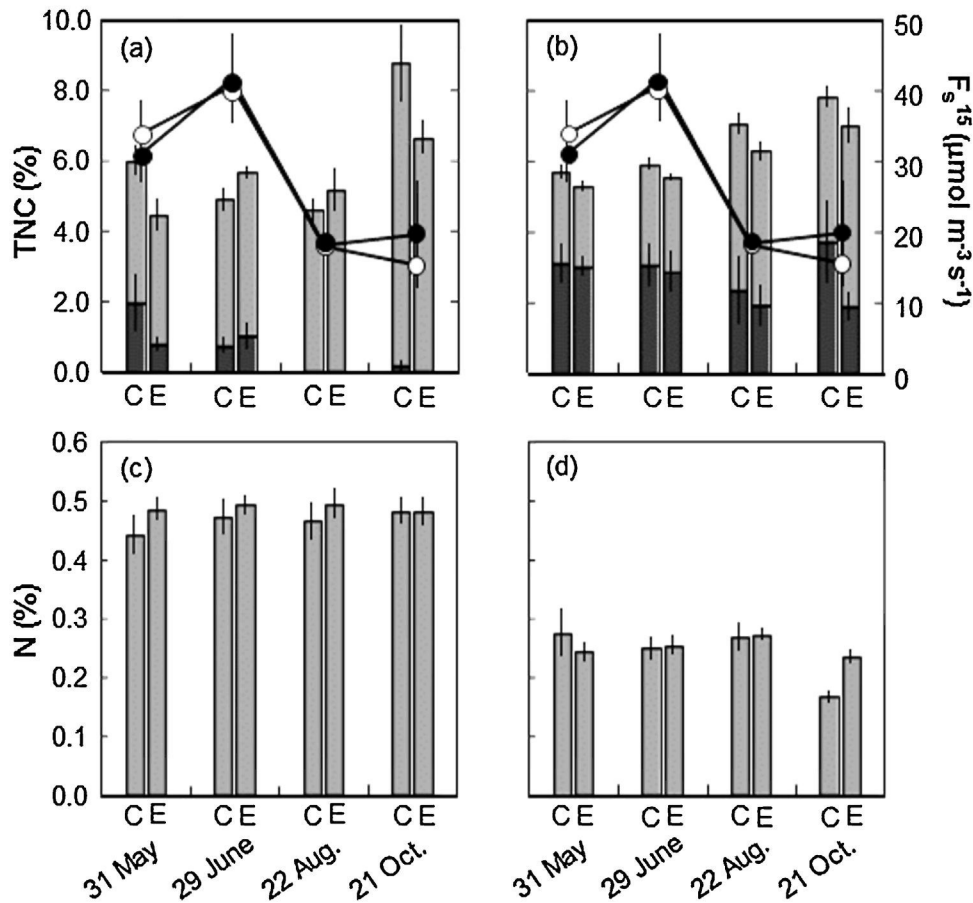
## 4. Discussion

### 4.1. Diurnal changes in stem CO<sub>2</sub> efflux

Temperature exerted a strong control on diel shifts of  $F_s$ . However, at a given temperature, daytime  $F_s$  was lower than the value



**Fig. 8.** Relationship between stem CO<sub>2</sub> efflux at 15°C ( $F_s^{15}$ ) and the rate of stem diameter increment. Data points correspond to individual stems in May (circles) and early August (triangles) subjected to either normal rainfall (white symbols) or partial throughfall exclusion (black symbols). Values of  $F_s^{15}$  are expressed as a function of stem surface area. A regression model was depicted for all data pooled ( $y = 0.70 + 0.52x$ ;  $r^2 = 0.51$ ,  $n = 20$ ,  $P < 0.001$ ).



**Fig. 9.** Means ( $\pm$ SE) of nonstructural carbohydrates (TNC), nitrogen (N) and sapwood-based stem CO<sub>2</sub> efflux at 15°C ( $F_s^{15}$ ) in different dates of 2011. Left panels (a, c) refer to TNC and N concentrations in the inner bark, and right panels (b, d) to sapwood concentrations. Circles refer to  $F_s^{15}$ . Means are for five trees subjected to either normal rainfall (C; white circles) or partial throughfall exclusion (E; black circles). The concentration of TNC is separated into that of soluble sugars (upper bars) and starch (lower bars).

expected from the relationship of  $F_s$  vs.  $T_s$  at  $V_s < 0.01 \text{ mm s}^{-1}$ . Increasing  $V_s$  caused an increasing depression of daytime  $F_s$  in most dates and, on average,  $F_s$  standardized at 15°C ( $F_s^{15}$ ) was 7% lower when it was estimated from all data than when it was estimated from only data of  $F_s$  vs.  $T_s$  at  $V_s < 0.01 \text{ mm s}^{-1}$ . Based on similar findings, Teskey et al. (2008) stated that part of the CO<sub>2</sub> respired by the cells in the stem is transported dissolved in the sap towards the leaves. Here the effect of sap flow in depressing  $F_s$  disappeared or was reversed when the delay in the response of  $F_s$  to temperature was taken into account. The same result was found by Maier

and Clinton (2006). By regressing  $F_s$  against preceding stem temperatures we could have artefactually removed the effect of  $V_s$  in transporting respired CO<sub>2</sub> upward. But it could well be the case that CO<sub>2</sub> diffused radially at a slower pace than air and stem temperatures increased during the day. Slow CO<sub>2</sub> radial diffusion can cause a lagged response of  $F_s$  to temperature and be responsible for its diel hysteresis (Ryan et al., 1995). Low  $V_s$  imposed by climatic conditions and small-diameter xylem vessels of *Q. ilex*, particularly in this dry region (Villar-Salvador et al., 1997), could make restrictions in CO<sub>2</sub> radial diffusion to be more relevant for diel shifts in  $F_s$  than  $V_s$  itself, as concluded by Cerasoli et al. (2009).

The lag of  $F_s$  relative to stem temperature tended to be longer and occur in more trees as drought increased. A similar result was observed by Saveyn et al. (2007b) in *Quercus robur* trees subjected to a short cycle of drought. The radial diffusion of CO<sub>2</sub> depends on the gradient of CO<sub>2</sub> concentration between the atmosphere and the stem, and the barriers to CO<sub>2</sub> diffusion imposed by the different stem tissues. Increased lignification and thickening of cells could have increased the resistance to CO<sub>2</sub> diffusion through wood and bark at the end of summer (Stockfors and Linder, 1998; Teskey et al., 2008); however, this would not explain why the number of trees showing a lagged response of  $F_s$  to temperature decreased after autumn rains, since anatomical changes are not rapidly reversible. Saveyn et al. (2007b) found that the onset of drought brought about a decline in the xylem CO<sub>2</sub> concentration, and Teskey and McGuire (2005) found that xylem CO<sub>2</sub> concentration was positively related with  $F_s$ . Based on these results we hypothesize that as a consequence of drought, respiration (see next section) and the concentration of CO<sub>2</sub> in the xylem decreased, which slowed down CO<sub>2</sub>

**Table 2**

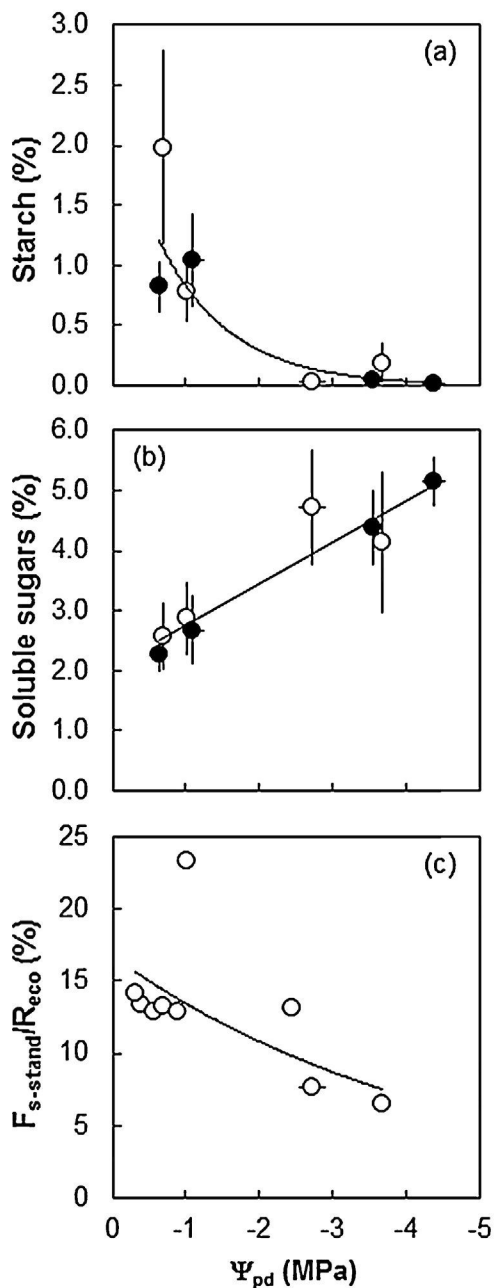
Daily stem CO<sub>2</sub> efflux per unit ground area ( $F_{s\text{-stand}}$ ;  $\text{g C m}^{-2} \text{ day}^{-1}$ ), ecosystem respiration ( $R_{\text{eco}}$ ;  $\text{g C m}^{-2} \text{ day}^{-1}$ ) and proportion of  $R_{\text{eco}}$  attributed to  $F_{s\text{-stand}}$  ( $F_{s\text{-stand}}/R_{\text{eco}}$ ; %), at different dates of 2011.

Date	$F_{s\text{-stand}}^a$	$F_{s\text{-stand}}^b$	$R_{\text{eco}}$	$F_{s\text{-stand}}^a/R_{\text{eco}}$	$F_{s\text{-stand}}^b/R_{\text{eco}}$
9 March	0.21	0.18	1.59	13.37	10.98
20 April	0.38	0.29	2.97	12.92	9.82
31 May	0.41	0.37	3.06	13.24	12.17
29 June	0.68	0.42	2.93	23.34	14.26
2 August	0.46	0.39	3.58	12.88	11.00
22 August	0.37	0.50	4.82	7.68	10.32
23 September	0.27	0.36	2.08	13.17	17.51
21 October	0.10	0.22	1.49	6.54	14.73
24 November	0.38	0.20	2.68	14.11	7.31

<sup>a</sup> Values of stem CO<sub>2</sub> efflux per unit sapwood volume at 15°C ( $F_s^{15}$ ) and  $F_s$  temperature sensitivity ( $Q_{10}$ ) derived from the specific temperature response curves of  $F_s$  of each date were used to calculate  $F_{s\text{-stand}}$  for each date.

<sup>b</sup> Values of  $F_s^{15}$  and  $Q_{10}$  derived from the annual response curve of  $F_s$  to temperature were used to calculate  $F_{s\text{-stand}}$  for each date.



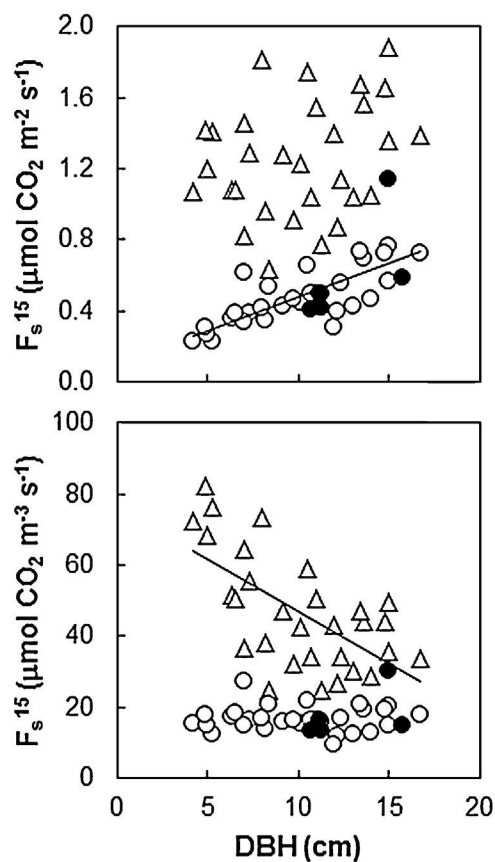


**Fig. 10.** Relationship between (a) concentration of starch in the inner bark, (b) concentration of soluble sugars in sapwood, and (c) proportion of ecosystem respiration attributed to stem  $\text{CO}_2$  efflux per unit ground area ( $F_{s\text{-stand}}/R_{\text{eco}}$ ) with shoot predawn water potential ( $\Psi_{\text{pd}}$ ). Values in (a) and (b) are means ( $\pm 2\text{SE}$ ) of  $n=4-7$  stems among dates subjected to either normal rainfall (white symbols) or partial throughfall exclusion (black symbols). Significant regression models were depicted for all data pooled: (a)  $y = 2.53e^{0.94x}$ ;  $r^2: 0.73$ ,  $n=8$ ,  $P<0.01$ ; (b)  $y = 2.07 - 0.69x$ ;  $r^2: 0.89$ ,  $n=8$ ,  $P<0.001$ ; and (c)  $y = 16.34e^{0.17x}$ ;  $r^2: 0.33$ ,  $n=9$ ,  $P<0.001$ .

radial diffusion in our study. Similarly, lower sapwood in smaller stems could make for lower  $\text{CO}_2$  concentration and radial diffusion than in bigger ones, which would explain why smaller trees, despite their lower path for  $\text{CO}_2$  diffusion, showed more often a lagged response of  $F_s$  to temperature than bigger ones.

#### 4.2. Seasonal changes in stem $\text{CO}_2$ efflux

Temperature and water availability affected  $F_s$  through the control they exerted on growth phenology and maintenance  $R_s$ . Here the estimation of growth and maintenance components of



**Fig. 11.** Relationship between stem  $\text{CO}_2$  efflux at  $15^\circ\text{C}$  ( $F_s^{15}$ ) expressed per unit of (a) surface area or (b) sapwood volume with diameter at breast height (DBH). Data points are individual stems measured in April (triangles) and late August (circles). Black symbols depict the five trees subjected to throughfall exclusion. Significant regression models were depicted in: (a)  $y = 0.10 + 0.04x$ ;  $r^2: 0.51$ ,  $n=35$ ,  $P<0.001$  both treatments pooled; and (b)  $y = 76.2 - 2.93x$ ;  $r^2: 0.40$ ,  $n=30$ ,  $P<0.001$ .

$R_s$  at each date was problematic. One explanation is that in most dates  $F_s$  reflected respiratory costs associated to both growth and maintenance, even when significant stem diameter increments were not observed. Long lasting processes related with growth that go undetected by dendrometer bands, such as cell-wall lignification (Sprugel and Benecke, 1991; Gindl et al., 2000), could complicate the separation of growth and maintenance respiratory components (Lavigne et al., 2004). It could also explain why  $F_s$  (standardized at  $15^\circ\text{C}$ ) did not peak at the same time than radial growth. Several authors have discussed high  $F_s$  following periods of stem diameter increment reflects the high demand of respiratory products during the processes of cell wall thickening and lignification (Ryan, 1990; Sprugel and Benecke, 1991; Cannel and Thornley, 2000; Edwards and Hanson, 1996; Gaumont-Guay et al., 2006). By assuming that only the lowest value of  $F_s^{15}$  observed over the year reflected exclusively maintenance costs, the calculated growth component of  $R_s$  showed a clear seasonal pattern. However, it indicated growth  $R_s$  accounted for 80% of total  $F_s$  in March, two months before any change in diameter was registered by dendrometers. Although cambial reactivation has been adduced to explain high  $F_s$  before growth is observed (Rambal et al., 2004; Gaumont-Guay et al., 2006; Gruber et al., 2009), it is more likely that maintenance  $R_s$  acclimated to low temperatures and caused high rates of  $F_s$  in March (Paembonan et al., 1991; Gansert et al., 2002; Atkin and Tjoelker, 2003). A number of mechanisms required to maintain stem functionality during winter might up regulate the respiratory metabolism (Cavender-Bares, 2005). Seasonal acclimation of maintenance  $R_s$  to temperature and water stress

can affect the partitioning of  $R_s$  into growth and maintenance in climatic regions with ample seasonal variations in temperature and soil water availability.

Thus, the drought-induced decline in  $F_s$  was likely caused by a progressive reduction in the demand of energy from growth and maintenance processes. Low turgor pressure of living cells, reflected in the contraction of stem diameter during the drought (see Zweifel et al., 2006; Fig. 7), could also be one factor underpinning low  $F_s$  during this period, as adduced by Saveyn et al. (2007a,b). On the contrary, drought-induced variations in  $V_s$  could affect the concentration of oxygen in the stem, but less likely  $R_s$  because the threshold for oxygen inhibition of mitochondrial respiration is very low (Spicer and Holbrook, 2007). Data on non-structural carbohydrates (TNC) suggested that soluble sugars (SS) did not limit  $R_s$  during the dry period either. As previously found by Larcher and Thomas-Thin (1988) in *Q. ilex*, SS tended to increase in dry dates, to some extent at the cost of starch in the inner bark. The rise in the concentration of SS may increase the osmoticum in the cells and help to maintaining phloem carbon transport during the period of higher water stress (Sevanto et al., 2014).

#### 4.3. Does stem respiration acclimate to nine years of experimentally increased drought?

One objective of this work was to assess whether a sustained, long-term reduction in rainfall resulted in a change in  $F_s$ . Over the course of the experiment,  $Q_{10}$  and  $F_s$  never significantly differed between *C* and *E* trees, suggesting  $R_s$  remained unaffected by the throughfall exclusion. A similar result was found for leaves of *Q. ilex* (Rodríguez-Calcerrada et al., 2011), which supports the idea that long-term acclimation to drought relies on the adjustment of morphological traits at the whole-tree level (Martin-StPaul et al., 2013). Only one stem from the *E* treatment showed increasingly higher  $F_s$  rates than the rest as drought progressed, up to 4 times higher at the peak of drought, which made for a non-significant trend of higher  $F_s$  in *E* than *C* trees at low  $\Psi_{pd}$ . Although *E* trees achieved lower growth than *C* trees over the entire year, stem growth rate was similar between treatments around the time of  $F_s$  measurements. This and the similar growth respiration coefficient contributed to explain why  $F_s$  was the same in *C* and *E* trees during the period of stem growth. The similar growth respiration coefficient among treatments ( $0.19 \text{ g C respired g}^{-1} \text{ C wood formed}$ , see also Rambal et al. (2004)) is consistent with the observation that construction costs are relatively constant among plants with contrasted growth conditions (Cannel and Thornley, 2000). Furthermore, similar rates of  $F_s$  during the period with no radial growth point out to similar metabolic costs to maintaining living stem tissues, which is consistent with the similar wood density and concentrations of non-structural carbohydrates and nitrogen in both treatments.

Two considerations should be borne on mind on the comparison of  $F_s$  between *C* and *E* trees. Firstly, differences in  $V_s$  between treatments were of little relevance for  $F_s$ , given the low influence of  $V_s$  on  $F_s$ . Indeed, the comparison of night-time  $F_s$  between treatments led to the same suggestion that  $R_s$  was not affected by the throughfall exclusion. Secondly, stem cores were not taken in the trees sampled for  $F_s$  because of the long-term nature of this experiment. Sapwood-based rates of  $F_s$  were thus calculated assuming the same allometric relationship between stem diameter and sapwood area held for both treatments.

#### 4.4. Stand-level stem $\text{CO}_2$ efflux

The average release of  $\text{CO}_2$  from stems ( $F_{s\text{-stand}}$ ) amounted to  $0.36 \text{ g C m}^{-2} \text{ soil day}^{-1}$  and was equivalent to 13% of all  $\text{CO}_2$  released

from the ecosystem; to the best of our knowledge this is the first estimate for a Mediterranean evergreen broadleaf forest. The contribution of  $F_{s\text{-stand}}$  to ecosystem  $\text{CO}_2$  efflux ( $R_{\text{eco}}$ ) varied from 6.5 to 23.3% over the year. It was the highest after spring radial growth and decreased with increasing water stress, probably because of lower sensitivity of  $R_s$  to fluctuations in topsoil moisture than the rest of ecosystem components. Soil respiration is the most important source of  $\text{CO}_2$  in the ecosystem and is very sensitive to rain pulses (Joffre et al., 2003; Allard et al., 2008). In fact, in forests where trees have access to deep soil water, soil respiration seems more sensitive to summer rainfall shortage and small rain events than aboveground respiration (Reichstein et al., 2002; Joffre et al., 2003; Wieser et al., 2009; Guidolotti et al., 2013). Thus, frequent events of rainfall during summer probably boosted soil respiration and thus  $R_{\text{eco}}$ , but had a lower effect on  $R_s$ .

Respiration of ecosystem components at fine time scales (minutes to days) is in many cases estimated from annual curves of respiration vs. temperature. However, the base rate of respiration and its temperature sensitivity can change over the year. Here, disregarding the seasonal variation of  $F_s^{15}$  that took place mainly in relation to tree water availability led to an overestimation of  $F_{s\text{-stand}}$  that ranged from 33 to 125% during the drought period, and an underestimation of  $F_{s\text{-stand}}$  from 8 to 48% in the rest of dates. The data suggest that  $F_{s\text{-stand}}$  and its contribution to  $R_{\text{eco}}$  and net carbon exchange should be estimated after accounting for seasonal changes in respiratory characteristics, particularly in Mediterranean-type forest ecosystems.

Some assumptions were made on the scaling up that can be further refined. First, we scaled up rates of  $F_s$  at DBH to the entire stem, while it has been reported that specific rates of respiration vary along the stem (e.g. Damesin et al., 2002; Pruyn et al., 2002). Second, in April, sapwood-based  $F_s$  rates were negatively correlated with stem diameter – suggesting that smaller and perhaps younger stems had a higher cambial activity than larger ones (Bosc et al., 2003). Based on this result,  $F_s^{15}$  was considered to vary between trees as a function of their DBH in spring (i.e. both in April and May). Higher surface-area-based  $F_s$  of bigger than smaller stems in summer disappeared when  $F_s$  was expressed per sapwood volume. This indicated  $F_s$  in summer was mostly dependent on stem sapwood volume (Edwards and Hanson, 1996), and that the proportion of living cells in the xylem was rather constant across the observed range of stem diameter.

#### 4.5. Conclusions

Stem  $\text{CO}_2$  efflux varied seasonally, not in relation with sap flow or the availability of carbohydrates, but likely in relation with a temperature- and drought-induced variation in the demand of respiratory products. This calls to be cautious when extrapolating modelled rates of stem  $\text{CO}_2$  efflux from annual temperature response curves to shorter time scales. The strong relationship between seasonal changes in tree water availability and stem  $\text{CO}_2$  efflux could be useful for modelling purposes in ecosystems with no seasonality in stem growth. Nine years of continuous throughfall exclusion did not affect stem  $\text{CO}_2$  efflux and its sensitivity to environmental factors, which suggest there was no acclimation of stem respiration to a long-term increase in drought intensity.

#### Conflict of interest

The authors declare no conflict of interest in relation with this work.



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